JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2010, p. 3582–3592 Vol. 48, No. 10
0095-1137/10/$12.00 doi:10.1128/JCM.00657-10

Virulence Genes and Genotypic Associations in Nasal Carriage, Community-Associated Methicillin-Susceptible and Methicillin-Resistant USA400 *Staphylococcus aureus* Isolates▽†

Sanjay K. Shukla,[1]* Mary E. Karow,[2] Jennifer M. Brady,[1] Mary E. Stemper,[1] Jennifer Kislow,[1] Natalie Moore,[2] Katherine Wroblewski,[2] Po-Huang Chyou,[1] David M. Warshauer,[3] Kurt D. Reed,[4] Ruth Lynfield,[5] and William R. Schwan[2]

Marshfield Clinic Research Foundation, Marshfield, Wisconsin[1]; University of Wisconsin—La Crosse, La Crosse, Wisconsin[2]; Wisconsin State Laboratory of Hygiene, Madison, Wisconsin[3]; Feinberg School of Medicine, Northwestern University, Chicago, Illinois[4]; and Minnesota Department of Public Health, St. Paul, Minnesota[5]

Received 29 March 2010/Returned for modification 18 May 2010/Accepted 20 July 2010

It is not well understood why strains of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), a major cause of skin and soft tissue infections, became successful so quickly, overtaking the place of methicillin-sensitive *S. aureus* (MSSA) in many communities. To evaluate the genetic basis of differences in their virulence traits, 293 *S. aureus* isolates consisting of three cohorts, genotypically defined clinical CA-MRSA ($n = 77$), clinical MSSA ($n = 103$), and nasal carriage MSSA ($n = 113$), collected over a 19-year period in two Midwestern states in the United States, were (i) extensively genotyped and (ii) screened for 40 known virulence genes which included those for enterotoxins, leukocidins, hemolysins, and surface proteins and several newly identified putative toxin genes from the USA400 lineage of CA-MRSA. Genotypically, nasal carriage and clinical MSSA isolates were much more diverse than was the CA-MRSA group, which was found to be of USA400 lineage only. Virulence gene profiles of the three groups showed that CA-MRSA strains harbored significantly higher percentages (≥95%; *P* value, <0.05) of the *sea*, *sec*, *sec4*, *seg2*, *seh*, *sek*, *sel*, *sel2*, *ear*, *ssl1*, *lpl10*, *lukSF-PV*, *lukD*, *lukE*, and *clfA* genes than did the carriage and the clinical MSSA group (range, 0% to 58%). Genes of the enterotoxin gene cluster, *seg*, *sei*, *sem*, *sen*, and *seo*, were present in the clinical and carriage isolates but not in the CA-MRSA group. These results suggest that the presence of additional virulence factors in USA400 CA-MRSA strains compared to the nasal carriage and clinical MSSA strains probably contributed to their enhanced virulence.

Staphylococcus aureus both is a benign commensal and common pathogen in humans and is responsible for a variety of infections, ranging from superficial skin and soft tissue infections to bacteremia, endocarditis, and osteomyelitis (33). Based on its susceptibility to beta-lactams, *S. aureus* is commonly described as methicillin-susceptible *S. aureus* (MSSA) or methicillin-resistant *S. aureus* (MRSA) (15). Infections due to health care-associated MRSA (HA-MRSA) have been a problem since the 1970s; however, starting in the 1990s, new strains of MRSA, referred to as community-associated MRSA (CA-MRSA), appeared in community dwellers and were genetically different from HA-MRSA strains (7). Until recently, individuals who presented with infections due to CA-MRSA typically have had none of the established risk factors associated with HA-MRSA, such as recent hospitalization, surgery, dialysis, long-term care residence, or indwelling medical devices. Lately however, CA-MRSA strains have been reported from both the community and the health care settings (25, 45). Genotyping tools such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and *spa* typing have helped in distinguishing the genotypes of CA-MRSA strains from those of other *S. aureus* strains (2, 14, 26). In PFGE, SmaI-restricted *S. aureus* genomes are compared to determine their genetic relatedness and also compared against the reference USA genotypes (USA100, USA200, etc., up to USA1200) as described by the Centers for Disease Control and Prevention (CDC) (34, 53). Of these, CA-MRSA isolates mostly belong to USA300 and USA400 clones and in some cases USA1000 and USA1100 clones as well. HA-MRSA isolates generally belong to USA100, USA200, and USA500 (34, 35). One of the two major clones of CA-MRSA, USA400, recognized in the early 1990s and initially referred to as the MW2 clone, was the predominant CA-MRSA clone that initially circulated in the midwestern United States in the 1990s (8, 17, 40, 52). The second and more recent CA-MRSA clone, USA300, was first recognized in 2000 and has since spread throughout the world (54). More than a thousand MLST allelic profiles for *S. aureus* have been identified so far, of which CA-MRSA strains are primarily represented by sequence type 1 (ST1) (USA400) and ST8 (encompasses USA300 and USA500). Of the several thousand *spa* types in the Ridom database (http://spaserver.ridom.de/), the most predominant CA-MRSA *spa* types are t008 and t128.

CA-MRSA strains, besides their distinct PFGE, ST, and *spa* profiles, almost ubiquitously possess the Panton-Valentine leukocidin (PVL or *lukSF-PV*) genes, in contrast to only 1% to 5%

* Corresponding author. Mailing address: Molecular Microbiology Laboratory, Marshfield Clinic Research Foundation, 1000 North Oak Avenue-MLR, Marshfield, WI 54449. Phone: (715) 389-5363. Fax: (715) 389-4950. E-mail: shukla.sanjay@mcrf.mfldclin.edu.

† Supplemental material for this article may be found at http://jcm.asm.org/.

▽ Published ahead of print on 28 July 2010.

of *S. aureus* strains overall having these genes (33, 50, 57). The PVL toxin has been implicated in many skin and soft tissue infections and lethal necrotizing pneumonia, but the exact role of PVL during *S. aureus* infections remains controversial (10, 28, 31, 58). The genome sequence of the CA-MRSA strain MW2 of the USA400 lineage showed the presence of several additional putative toxin genes (e.g., *ear*, *sec4*, *sel2*, *seg2*, *ssl1* [*set16*], and *lpl10*) compared with other *S. aureus* strains that had been sequenced (1). Some of these toxin genes share homology with classic staphylococcal enterotoxin genes that encode pyrogenic exotoxins (49) typically produced during the post-exponential phase of growth, and the genes encoding these exotoxins are most often carried on plasmids, bacteriophages, or pathogenicity islands. The classic staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, and *seo*) are commonly found in strains of *S. aureus* (12, 23, 39, 42, 43, 46, 62). Pyrogenic exotoxin genes are common in *S. aureus*, and as many as 73% of *S. aureus* isolates carry at least one of the genes encoding a classic pyrogenic exotoxin; however, the distribution among various clonal types differs (3). Strains of USA400 CA-MRSA typically have been shown to possess the *sea*, *sec*, *seh*, and *sek* enterotoxin genes, whereas HA-MRSA strains usually carry the *sed*, *seg*, *sei*, *sej*, *sem*, *sen*, and *seo* enterotoxin genes (40). Currently, the distribution of the newly identified putative toxin genes (*seg2*, *sel2*, *sec4*, *ssl1*, and *lpl10*) (1) from the MW2 strain has not been reported from among CA-MRSA strains in general or from clinical and nasal carriage MSSA strains. Since CA-MRSA isolates are able to cause disease in humans without predisposing risk factors and have spread rapidly in communities, these strains may possess a greater number of toxin genes than do the other strains of *S. aureus*.

The aim of this study was to compare the genotypes of clinical CA-MRSA USA400, clinical MSSA, and colonizing nasal carriage MSSA isolates and determine the frequency and distribution of the classic enterotoxin genes as well as the new putative toxin genes in them. Our results showed that MSSA strains were much more diverse in their genotypes than were CA-MRSA USA400 strains. In addition, CA-MRSA USA400 strains possessed a distinct array of toxin genes compared to MSSA strains. These data may provide insight into the success of CA-MRSA USA400 and its ability to cause severe disease in previously healthy people.

MATERIALS AND METHODS

***S. aureus* strains.** Two hundred ninety-three *S. aureus* isolates collected over a 19-year period from 1987 to 2005 from outpatient and surveillance cultures from Wisconsin and the Minnesota Department of Health were included in the study. The collection periods for the three groups of the isolates differed but did overlap. These isolates were classified into three groups, (i) nasal carriage MSSA ($n = 113$), (ii) clinical MSSA ($n = 103$), and (iii) clinical CA-MRSA ($n = 77$), based on the collection and phenotypic criteria (Table 1). Sixty-six percent of nasal carriage strains were from Wisconsin and were collected from college-going healthy individuals. Anterior nares of these individuals were screened for *S. aureus* followed by determination of their resistance to methicillin by MIC testing using oxacillin. Putative MRSA strains were then confirmed using growth on cation-adjusted Mueller-Hinton II agar plates containing 6 μg/ml of oxacillin. None of the subjects were recently hospitalized or had exposure to health care environments. Thirty-four percent of the nasal colonization isolates were from the Minnesota Department of Health and were from a collection of MSSA isolates obtained from outpatients in the upper Midwest. Clinical MSSA strains were strains recovered from patients with confirmed disease due to an MSSA strain. They were mainly recovered from skin and soft tissue infections and in rare instances from invasive cases such as blood. The CA-MRSA strains were primarily (>90%) isolated from a variety of skin and soft tissue infections from outpatient clinics and were genotypically defined. gCA-MRSA strains in this study were strains whose PFGE was related to the MW2 PFGE type and harbored staphylococcal chromosome cassette *mec* (SCC*mec*) IV. In addition they were recovered from patients visiting outpatient clinics.

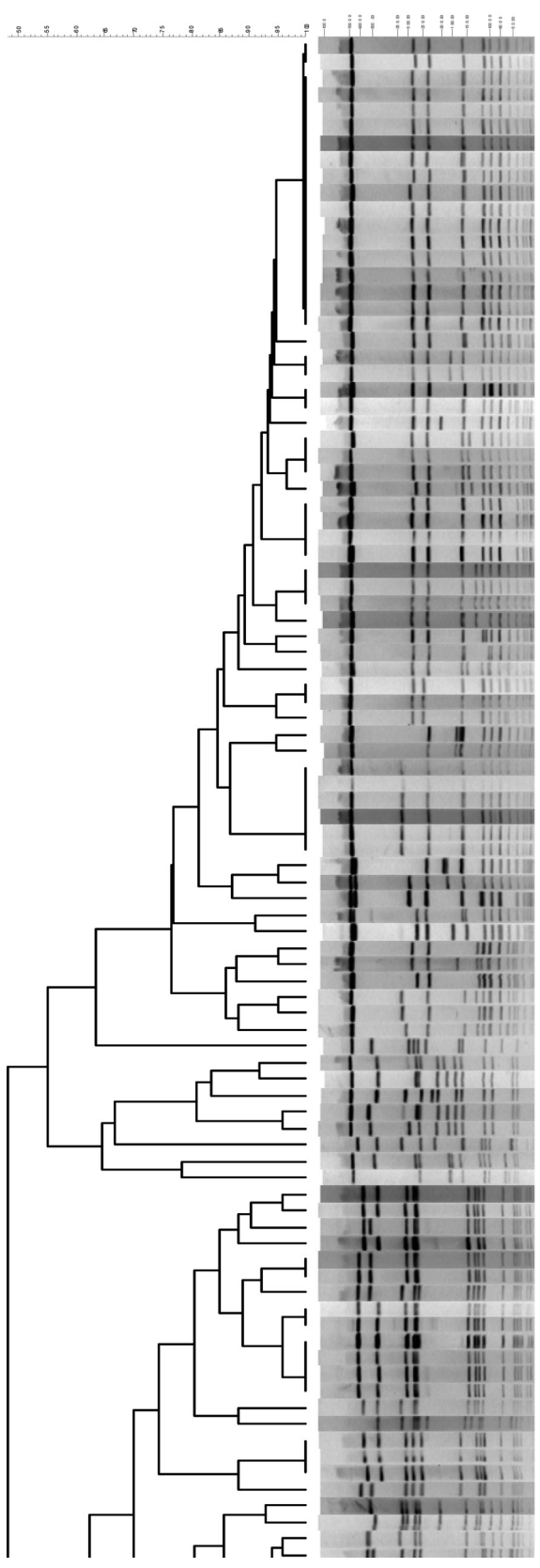
TABLE 1. Grouping of 293 *S. aureus* isolates studied and their years of collection

Group	Collection period	No. of isolates	Collection site(s)
Clinical MSSA	1987–2006	103	WI, MN
Nasal carriage MSSA	2000–2005	113	Upper Midwest
CA-MRSA	1990–1999	77	WI

Genotypic methods. All isolates were typed by PFGE, MLST, and *spa* typing (2, 14, 26). SCC*mec* types were determined for MRSA isolates by the method of Oliveira and de Lencastre (41). PFGE clonal groups were defined by the Tenover et al. (53) criteria and ≥80% genetic similarity using the Dice coefficient (1.25% tolerance) and UWPGMA (unweighted-pair group method using average linkages) method. An MLST-based clonal complex (CC) was defined as a group that shared six of the seven MLST alleles through the use of eBURST (http://eburst.mlst.net).

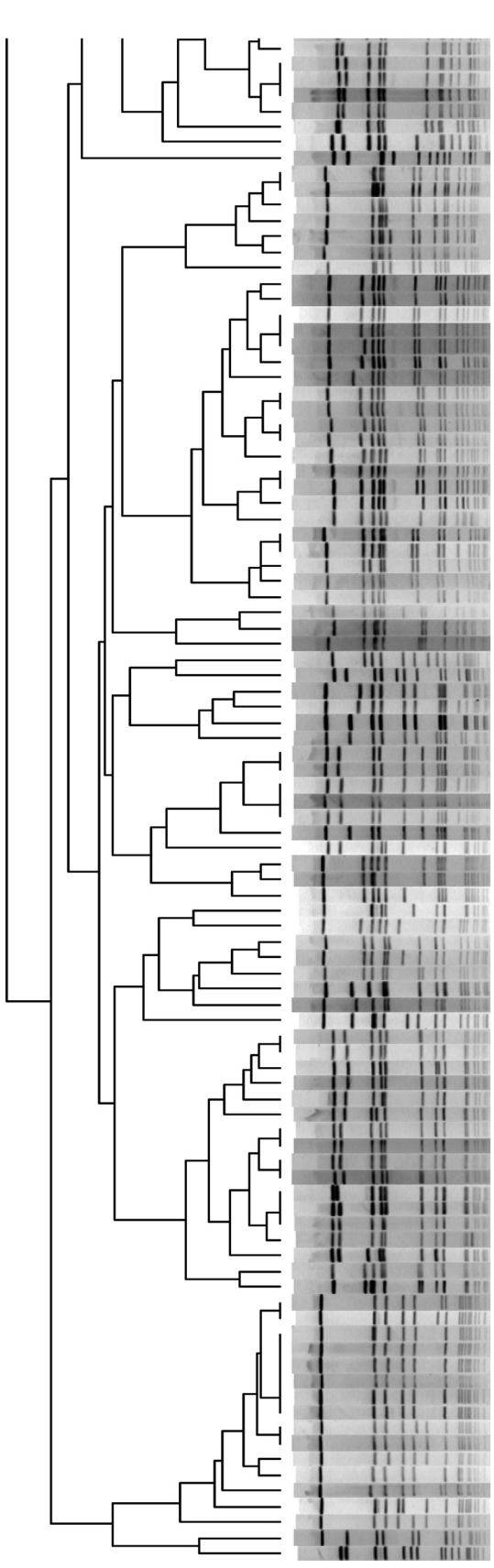
Screening for virulence factor genes. All isolates were screened for 40 known and putative *S. aureus* virulence genes located on bacteriophages and pathogenicity and genomic islands on the *S. aureus* genome. Of the 40 virulence genes, the following have been shown to have clinical relevance: classical staphylococcal enterotoxin genes (*sea* to *see*), the toxic shock syndrome toxin gene (*tst*), enterotoxin gene cluster or *egc* (*seg*, *sei*, *sem*, *sen*, and *seo*), the collagen binding protein gene (*cna*), fibronectin binding protein genes (*fnbA* and *fnbB*), clumping factor genes (*clfA* and *clfB*), genes for the serine-aspartate repeat family of proteins (*sdrC*, *sdrD*, and *sdrE*), the intracellular adhesion protein gene (*icaA*), hemolysin genes (*hla*, *hlb*, *hld*, and *hlgB*), leukocidin genes (*lukSF-PV*, *lukD*, and *lukE*), and exfoliative toxin genes (*eta* and *etb*). Additionally seven putative virulence genes (*ear*, *sec4*, *sel2*, *bsa*, *seg2*, *lpl10*, and *ssl1*) were screened because of their speculated role in *S. aureus* virulence (1). The presence or absence of these genes was screened by a combination of singleplex or four multiplex PCRs. The list of primers for the virulence genes, their locations on the genome, and expected product sizes are given in Table S1 in the supplemental material. The PCR primers were commercially synthesized at Integrated DNA Technology (Coralville, IA). All the control strains were obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA). The multiplex PCRs included multiplex 1 (*sea* to *see*), multiplex 2 (*seg* to *sej*), multiplex 3 (*sek* to *seo*), and multiplex 4 (*seg2*, *sec4*, and *ear*). Singleplex PCRs were run for the remainder of the genes. Using the genomic DNA as templates, PCR was performed using the HotStarTaq Master Mix kit (Qiagen, Valencia, CA). The *sea*-to-*see* multiplex reaction mixtures were set up in a 50-μl PCR mixture and consisted of 25 μl of the HotStarTaq Master Mix; 40 pmol of each of the forward and reverse primers for *sea* and *seb*; 10 pmol of each of the forward and reverse primers for *sec*, *sed*, and *see*; 3 μl of 25 mM Mg^{2+}; 7 μl of water; and 4 μl of the extracted DNA template. The *seg*-to-*sej* multiplex reaction mixture consisted of 25 μl of the HotStarTaq Master Mix; 10 pmol of each of the forward and reverse primers for *seg*, *seh*, *sej*, and *sei*; 0.5 μl of 25 mM Mg^{2+}; 16.5 μl of water; and 4 μl of the extracted DNA template. For *sek* to *seo*, the reaction mixture consisted of 25 μl of the HotStarTaq Master Mix; 10 pmol of each of the forward and reverse primers for *sek*, *sel*, *sem*, *sen*, and *seo*; 1.0 μl of 25 mM Mg^{2+}; 15 μl of water; and 4.0 μl of the template. The first three multiplex reaction mixtures went through an initial activation at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 2 min, annealing at 57°C for 2 min, and extension at 72°C for 1 min. A final extension was carried out at 72°C for 7 min followed by a final hold of 4°C. A PCR mix for multiplex PCR 4 included the HotStarTaq Master Mix kit, sterile double-distilled water, 50 pmol of *seg2*, 40 pmol each of *sec4* and *ear* primers, <1 μg of DNA template to the master mix that contained 2.5 U of HotStar *Taq* DNA polymerase, and 3.0 mM $MgCl_2$. The cycling conditions consisted of 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min.

At least two separate PCR amplifications per gene were performed for each *S. aureus* isolate to ensure reproducibility. The PCR products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide to visualize the DNA



Strain	PT	CG	ST	*spa*	CC
UWL-94-S	B1	B	t018	30	30
WSLH-43-S	B1	B	t363	30	30
MC-146-S	B1	B	t018	30	30
MC-133-S	B1	B	t012	30	30
MC-116-S	B1	B	t840	30	30
MC-151-S	B1	B	t1333	543	30
UWL-13-S	B1	B	t275	30	30
UWL-32-S	B1	B	t012	30	30
UWL-79-S	B1	B	t046	30	30
UWL-87-S	B2	B	t2920	30	30
WSLH-9-S	B1	B	t017	30	30
WSLH-19-S	B1	B	t2926	30	30
WSLH-10-S	B1	B	t021	30	30
WSLH-11-S	B1	B	t871	1101	30
WSLH-16-S	B1	B	t122	30	30
WSLH-30-S	B1	B	t1522	1103	30
WSLH-31-S	B1	B	t2923	30	30
WSLH-39-S	B1	B	t1642	30	30
UWL-39-S	B3	B	t012	30	30
MC-145-S	B5	B	t012	30	30
UWL-20-S	B5	B	t012	30	30
MNDH-107-S	B4	B	t2917	992	30
MC-140-S	B4	B	t012	30	30
USA200 (NRS383)			t018	36	
UWL-45-S	B7	B	t021	243	30
WSLH-1-S	B7	B	t1306	30	30
WSLH-22-S	B7	B	t012	30	30
WSLH-15-S	B6	B	t2910	30	30
UWL-89-S	B9	B	t012	30	30
WSLH-14-S	B9	B	t012	30	30
WSLH-34-S	B9	B	t338	1104	30
WSLH-38-S	B9	B	t018	30	30
UWL-9-S	B10	B	t012	30	30
UWL-23-S	B10	B	t019	30	30
UWL-93-S	B10	B	t012	30	30
UWL-16-S	B11	B	t012	30	30
MC-108-S	B12	B	t012	1107	30
UWL-76-S	B13	B	t021	30	30
WSLH-33-S	B8	B	t021	30	30
MNDH-106-S	B14	B	t012	30	30
UWL-72-S	B14	B	t021	1001	30
MNDH-101-S	B15	B	t012	30	30
MNDH-112-S	B16	B	t012	30	30
UWL-19-S	B17	B	t018	30	30
MC-144-S	B18	B	t274	30	30
MNDH-37-S	B18	B	t012	30	30
MNDH-47-S	B18	B	t1152	30	30
UWL-12-S	B18	B	t021	30	30
UWL-66-S	B18	B	t338	30	30
UWL-80-S	B18	B	t018	30	30
MNDH-124-S	B19	B	t2959	39	30
WSLH-29-S	B20	B	t2643	39	30
WSLH-7-S	B21	B	t2924	39	30
MC-106-S	B23	B	t2918	30	30
USA1100 (NRS484)				30	
MNDH-105-S	L1	L	t136	991	30
WSLH-23-S	L2	L	t166	1102	30
MNDH-108-S	L3	L	t136	989	30
MNDH-120-S	L4	L	t2917	989	30
MNDH-122-S	L5	L	t2917	989	30
MNDH-121-S	L6	L	t2917	989	30
MNDH-98-S	R1	R	t1960	1021	1021
MNDH-62-S	J3	J	t159	51	121
WSLH-20-S	J4	J	t159	51	121
MNDH-84-S	Y1	Y	t1961	51	121
MNDH-32-S	J1	J	t159	51	121
MNDH-60-S	J2	J	t2155	121	121
UWL-102-S	Z1	Z	t518	50	50
UWL-69-S	zz1	zz	t375	1000	509
UWL-91-S	yy1	yy	t1977	22	22
UWL-11-S	C5	C	t084	15	15
WSLH-36-S	C6	C	t346	15	15
UWL-84-S	C7	C	t335	15	15
WSLH-25-S	C8	C	t094	15	15
UWL-5-S	C3	C	t084	15	15
UWL-28-S	C3	C	t2925	15	15
MC-109-S	C4	C	t3027	15	15
MNDH-99-S	C2	C	t346	802	15
MC-101-S	C2	C	t084	15	15
MNDH-43-S	C1	C	t084	1143	15
UWL-26-S	C1	C	t084	15	15
UWL-74-S	C1	C	t774	15	15
UWL-88-S	C1	C	t2914	1003	15
UWL-24-S	C9	C	t084	15	15
UWL-95-S	C10	C	t316	59	15
MC-124-S	P1	P	t1164	9	9
UWL-50-S	P1	P	t2921	9	9
WSLH-21-S	P1	P	t193	9	9
MNDH-29-S	P2	P	t100	27	9
MC-132-S	G5	G	t216	59	59
WSLH-40-S	G6	G	t216	87	59
UWL-18-S	G2	G	t216	59	59
UWL-49-S	G3	G	t216	59	59

FIG. 1. PFGE-based dendrogram of *S. aureus* isolates consisting of at least one unique *spa* type from each of the three cohorts: clinical CA-MRSA, clinical MSSA, and nasal carriage MSSA. The dendrogram also includes PFGE-based USA genotypes as reference strains. The dendrogram was created using 1.25% tolerance, the Dice similarity coefficient, and the unweighted-pair group method using arithmetic averages. Clonal relatedness was determined by ≥80% genetic similarity. PT, pulsotype.



Strain	PT	CG	ST	*spa*	CC
UWL-49-S	G3	G	t216	59	59
UWL-30-S	G1	G	t216	59	59
UWL-77-S	G1	G	t316	59	59
WSLH-24-S	G1	G	t216	59	59
UWL-25-S	G4	G	t216	1108	59
MNDH-110-S	G7	G	t216	1110	59
USA1000 (NRS483)				59	
UWL-65-S	ww1	ww	t937	1112	398
MNDH-35-S	F4	F	t2909	1144	25
MNDH-94-S	F1	F	t078	25	25
MNDH-102-S	F2	F	t078	25	25
MNDH-103-S	F3	F	t078	25	25
MC-125-S	F6	F	t078	25	25
UWL-66-S	F5	F	t258	25	25
MNDH-115-S	F7	F	t660	1146	25
169-R	A5	A	t128	1	1
272-R	A2	A	t175	1	1
USA400 (MW2)			t125	1	1
34-R	A1	A	t128	1	1
88-R	A1	A	t128	1	1
397-R	A3	A	t128	1	1
33-R	A6	A	t128	1	1
MNDH-77-S	A7	A	t128	1	1
WSLH-6-S	A7	A	t558	1	1
MC-112-S	A8	A	t591	1	1
WSLH-32-S	A8	A	t922	1	1
MC-118-S	A9	A	t2915	1113	1
UWL-67-S	A16	A	t1178	1	1
UWL-83-S	A16	A	t922	1	1
WSLH-35-S	A17	A	t922	1	1
MNDH-97-S	A18	A	t160	1142	1
MC-127-S	A12	A	t2297	97	1
MNDH-125-S	A12	A	t189	97	1
UWL-35-S	A13	A	t267	97	1
UWL-92-S	A14	A	t359	97	1
MNDH-31-S	A15	A	t1965	987	1
MC-139-S	Q1	Q	t189	188	1
UWL-100-S	Q2	Q	t189	188	1
UWL-10-S	Q3	Q	t189	1106	1
WSLH-8-S	V1	V	t2913	1148	1148
WSLH-12-S	V2	V	t082	395	395
MNDH-36-S	M2	M	t2919	20	20
MNDH-25-S	M1	M	t164	20	20
WSLH-17-S	M3	M	t164	20	20
MC-177-S	M4	M	t164	20	20
MC-126-S	K2	K	t209	109	9
MC-167-S	K2	K	t209	109	9
MNDH-54-S	K1	K	t2922	1141	9
MC-131-S	K1	K	t209	109	9
UWL-41-S	K1	K	t209	109	9
WSLH-5-S	K3	K	t2700	144	9
USA100 (NRS382)			t002	5	
MNDH-90-S	S1	S	t2703	990	8
UWL-86-S	S2	S	t148	72	8
USA700 (NRS396)			t126	72	
USA800 (NRS397)			t088	5	
USA300 (NRS384)			t008	8	
MC-117-S	H2	H	t008	1150	8
UWL-78-S	H1	H	t404	30	30
MC-104-S	H4	H	t008	1105	8
UWL-37-S	H6	H	t622	8	8
UWL-99-S	H5	H	t334	8	8
USA500 (NRS395)			t064	8	
MC-115-S	D1	D	t067	5	5
UWL-33-S	D1	D	t179	5	5
UWL-81-S	D2	D	t062	5	5
UWL-85-S	D5	D	t179	5	5
MNDH-33-S	D3	D	t002	148	5
UWL-82-S	D6	D	t002	5	5
UWL-68-S	D7	D	t2916	5	5
UWL-4-S	D7	D	t548	5	5
MNDH-92-S	D8	D	t002	5	5
UWL-96-S	D8	D	t045	5	5
MNDH-46-S	D9	D	t688	148	5
MNDH-86-S	D9	D	t179	5	5
MNDH-85-S	D9	D	t067	5	5
MC-158-S	D10	D	t002	148	5
MNDH-69-S	D11	D	t002	5	5
MNDH-87-S	D13	D	t548	5	5
MNDH-113-S	D14	D	t548	5	5
UWL-27-S	E2	E	t015	45	45
UWL-67-S	E3	E	t065	45	45
MNDH-123-S	E1	E	t1549	45	45
MC-105-S	E1	E	t061	256	45
MC-138-S	E1	E	t2143	45	45
MC-174-S	E1	E	t2911	45	45
MNDH-82-S	E1	E	t1964	256	45
UWL-90-S	E1	E	t715	794	45
MC-128-S	E4	E	t004	45	45
MNDH-61-S	E4	E	t1826	45	45
MC-184-S	E5	E	t917	45	45
MNDH-70-S	E6	E	t1962	45	45
UWL-70-S	E7	E	t050	508	45
USA600 (NRS22)			t204	45	
UWL-36-S	E8	E	t330	45	45
MC-113-S	U1	U	t2912	395	395
WSLH-18-S	U2	U	t2927	395	395

FIG. 1—*Continued.*

bands. A 100-bp DNA ladder (New England Biolabs) was used to determine the size of each PCR product. All PCRs were done in a PE9700 Thermal Cycler (Perkin-Elmer, Wellesley, MA). A positive control for each respective gene and a negative control represented by a strain of *E. coli* were included for each run. A no-template control was also run for each PCR run to rule out the possibility of contamination of the reagents. A positive PCR was determined by the presence of an appropriately sized DNA band in the gel.

Identity of the amplicons. At least two randomly chosen amplicons for each of the 40 virulence genes tested were sequenced to ensure that primers amplified the expected genes. Gene identities were confirmed by comparison with the DNA sequence database at GenBank using the BLAST search.

Statistical analyses. All data analyses were carried out using the Statistical Analysis System (SAS, Cary, NC) and StatXact statistical software (Cytel Software Corp., Cambridge, MA). Fisher's exact test was used to derive the *P* value. Any *P* value less than 0.05 indicated statistical significance. Simpson's diversity index was determined as described previously (20).

RESULTS

Range of genotypic diversity in nasal carriage, clinical MSSA, and clinical CA-MRSA isolates. The nasal isolates (n = 113) were represented by 41 MLST allelic profiles or 15 clonal complexes (6 major and 9 minor). A major CC was defined as a CC that was represented by at least 4% of the isolates in that group. The six major CCs were CC30 (29.2%), CC15 (15%), CC5 (14.1%), CC25 (9.7%), CC59 (9.7%), and CC45 (5.3%). The remaining CCs were CC1, CC8, CC97, CC1021, CC12, CC22, CC50, CC398, and CC509, and their frequency ranged from 1% to 3.5%. These isolates were also represented by 68 unique PFGE types or pulsotypes that could be grouped into 18 PFGE-based clonal groups (CGs) using the criteria of Tenover et al. (53). The distribution of major CGs in order of their percentages was as follows: CG-B = CC30 or USA200 (23.9%), CG-C = CC15 (15.9%), CG-D = CC5 (12.4%), CG-F = CC25 (9.7%), CG-G = CC59 (8.8%), CG-A = CC15 or USA400 (5.3%), CG-E = CC45 or USA600 (5.3%), and CG-L = CC30 (4.4%). The frequencies of the remaining nine minor CGs were below 3%, and they were as follows: CG-H = CC8 and CC30, CG-K = CC9, CG-P = CC9, CG-Q = CC1, CG-R = CC1021, CG-S = CC8, CG-WW = CC398, CG-YY = CC22, CG-Z = CC50, and CC-ZZ = CC509. By our analysis, the non-USA types were represented by 65.5% of the isolates. Fifty-three *spa* types were identified, of which the seven major ones were t084 (10.6%), t012 (9.7%), t078 (8%), t216 (8%), t021 (4.4%), t548 (4%), and t2917 (3.5%).

Clinical MSSA isolates (n = 103) were represented by 38 MLST allelic profiles, which were grouped into 12 CCs. Seven major CCs were CC30 (39.8%), CC5 (14.6%), CC45 (8.7%), CC121 (7.8%), CC1 (5.8%), CC15 (4.9%), and CC20 (4.9%). The remaining CCs were CC59, CC395, CC8, CC25, and CC97, and their frequency ranged from 1% to 2.9%. This group was also represented by 59 pulsotypes which were grouped into 16 CGs. Three clones matched with the USA types: CG-B = CC30 or USA200 (38.8%), CG-A = CC-1 or USA400 (7.8%), and CG-E = CC45 or USA600 (5.8%). The non-USA types were represented by 47.6% of the isolates. Their breakdowns were as follows: CG-J = CC121 (6.8%), CG-D = CC5 (5.8%), CG-K = CC9 (5.8%), CG-C = CC15 (4.8%), and CG-M = CC20 (4.8%); the frequencies of the remaining 9 CGs (CG-F = CC25, CG-G = CC59, CG-H = CC8 and CC30, CG-L = CC30, CG-P = CC9, CG = Q = CC1, CG-U = CC395, CG-V = CC395 and CC1148, and CG-Y = CC121) were below 3%. Seventy-two *spa* types were identified in this group, with five major ones being t012 (13.6%), t159 (5.8%), t018 (4.9%), t164 (3.8%), and t209 (3.8%). The percent ranges of the remaining *spa* types were from 0.9% to 2.9%.

TABLE 2. Range of percentages of major CCs in three groups of isolates

CC	% of CC in isolate group:		
	Nasal carriage MSSA	Clinical MSSA	CA-MRSA
CC1	3.5	5.8	100
CC5	14.1	14.5	NF[a]
CC8	2.7	1.9	NF
CC12	0.9	NF	NF
CC15	15.0	4.9	NF
CC20	NF	4.9	NF
CC22	0.9	NF	NF
CC25	9.7	1.9	NF
CC30	29.2	39.8	NF
CC45	5.3	8.7	NF
CC50	0.9	NF	NF
CC59	9.7	2.9	NF
CC97	2.7	1.9	NF
CC121	NF	7.8	NF
CC395	NF	<2.9	NF
CC398	0.9	NF	NF
CC509	0.9	NF	NF
CC1021	2.7	NF	NF
Singletons	0.9	1	NF

[a] NF, not found.

The CA-MRSA isolates were the least diverse and were represented by nine PFGE types. However, they all belonged to CC1 (CG-A or USA400) but were still represented by six *spa* types: t128 (80.5%), t559 (6.5%), t125 (5.2%), t558 (5.2%), t175 (2.6%), and t555 (2.6%). Using the representative strains from each of the three groups, their comparative genotypic characteristics and diversity are summarized in Fig. 1. Overall, 18 CCs were identified in these three groups of isolates, of which CC20, CC121, and CC395 were not present in the nasal carriage group whereas CC12, CC22, CC50, C398, CC509, and CC1021 were absent in the clinical MSSA group (Table 2).

Nasal carriage and clinical MSSA strains were genetically more diverse by all three typing methods, unlike CA-MRSA isolates, which showed very little diversity by PFGE and MLST. Interestingly, there was no significant difference in the percentages of unique PFGE types, clonal groups, or MLST allelic profiles between the clinical MSSA strains and nasal carriage isolates. However, clinical MSSA strains were represented by significantly higher percentages of *spa* types than were the nasal carriage isolates. Overall, *S. aureus* isolates that were susceptible to methicillin were more diverse than were the MRSA isolates by PFGE. In terms of distinguishing abilities of the three typing methods, *spa* typing and PFGE were comparable but more discriminatory than MLST for clinical MSSA strains (Table 3). However, once PFGE types were grouped into clonal groups according to the Tenover et al. (53) criteria, *spa* typing was found to be more discriminatory. Clinical MSSA strains were found to have a higher percentage of *spa* types than were nasal carriage isolates. In addition to the simple descriptive analysis of diversity of genotypes described above, the diversity indices (DI) of nasal carriage and clinical

TABLE 3. Number of pulsotypes, PFGE-based clonal groups, STs, and *spa* types in study isolates collected during 1987 to 2005

Group (*n*)	No. (% diversity) of:			
	Pulsotypes	Clonal groups	STs	*spa* types
Nasal carriage MSSA (113)	71 (63)	18 (16)	41 (36)	52 (46)
Clinical MSSA (103)	59 (57)	17 (17)	38 (37)	64 (62)
CA-MRSA (77)	9 (12)	1 (1)	1 (1)	6 (7.8)

TABLE 4. Simpson's index of diversity of nasal carriage MSSA, clinical MSSA, and CA-MRSA isolates by PFGE, MLST, and *spa* typing

Genotypic method	Index of diversity for isolate group (*n*):	
	Nasal carriage MSSA (113)	Clinical MSSA (103)
spa typing	0.963	0.973
MLST	0.931	0.897
PFGE	0.988	0.946

MSSA isolates were 0.988 and 0.946 by PFGE, 0.963 and 0.973 by *spa* typing, and 0.931 and 0.897 by MLST, respectively (Table 4). Nasal carriage isolates were genotypically more diverse by MLST and PFGE than were clinical MSSA isolates.

Differential distribution of known virulence genes in CA-MRSA USA400 compared to clinical and nasal carriage MSSA isolates. Representative agarose gels of the four multiplex PCRs are shown in Fig. S1A to D in the supplemental material. Overall, the rate of positivity of the 40 virulence genes showed a wide range, from 0% to 100% across the three groups. Some genes were preferentially present in one group versus the two other groups. Twelve of the 40 genes (*ssl1*, *hla*, *hlb*, *hld*, *hlgB*, *clfB*, *cna*, *fnbA*, *icaA*, *sdrD*, *sdrE*, and *bsa*) were more frequently (≥85%) present across all three groups than were *sed*, *see*, *sej*, *etb*, and *fnbB*, which were present in the range of 0% to 24%. In addition, the rate of positivity of genes *seg*, *sei*, *sek*, *sel*, *sen*, *seo*, and *lukSF-PV* varied widely and ranged from <10% to >80% in the three groups.

Several enterotoxin genes (*sea*, *sec*, *sec4*, *seg2*, *sek*, *sel*, and *sel2*), leukocidin genes (*lukD*, *lukE*, and *lukSF-PV*), adhesion genes (*sdrD* and *clfA*), and putative virulence genes (*lpl10* and *ear*) were significantly associated with CA-MRSA compared to nasal carriage or clinical MSSA strains (*P* value of <0.05). Enterotoxin genes *sed* and *see*, including the enterotoxin gene cluster (*egc*), *seg*, *sei*, *sem*, *sen*, and *seo*; exotoxin genes (*tst*, *eta*, and *etb*); and the fibronectin binding protein gene (*fnbB*) were present in less than 1% of the CA-MRSA isolates. The toxic shock syndrome toxin gene (*tst*) and exfoliative toxin genes (*eta* and *etb*) were not present in the CA-MRSA group. Enterotoxin genes *sed* and *sej* were present at a low frequency, probably because the plasmid pIB485, which harbors these genes, may not be common in these groups of isolates. Genes *sea* and *sek*, which reside on ΦSa3mw, were almost always present in CA-MRSA isolates but infrequently present in MSSA isolates. Genes such as staphylococcal superantigen protein-like genes (*ssl1*), hemolysin genes (*hla*, *hlb*, *hld*, and *hlgB*), surface protein genes (*clfB*, *cna*, *fnbB*, *icaA*, and *sdrE*), and the bacteriocin gene (*bsa*) were present in nearly all three groups of the isolates. At the same time *fnbB*, although at a lower rate (14% to 24%), was present exclusively in MSSA strains.

In general, the MSSA clinical and nasal carriage strains have overall virulence profiles similar to those of the CA-MRSA group, except for *sel2*, *tst*, *hlb*, *cna*, *lpl10*, and *sdrC*, which were statistically different (*P* value, <0.05) when one was used as a reference group (Table 5). All of these genes except *lpl10* had a significantly higher rate of positivity in nasal carriage isolates than in the clinical MSSA strains. To address the issue of multiple comparisons, we applied the Bonferroni correction to data in Table 5 and found that the results for genes *sej*, *hlb*, *can*, *fnbA*, and *sdrE* were no longer statistically significant in each paired comparison among the three groups.

We also identified six nasal carriage and eight clinical MSSA isolates whose genotypes were similar to that of the USA400 clone. While the six nasal carriage MSSA strains were isolated in the year 2000 or later, two of the clinical MSSA strains (WSLH-6 and MNDH-77) in this group were recovered in 1987 and 1998, respectively, a period that either predated most of the CA-MRSA outbreaks or overlapped with the early outbreak. The virulence profiles of these 14 isolates were compared against those of the three representative CA-MRSA isolates of the USA400 lineage (Table 6). Notably, both WSLH-6 and MNDH-77 isolates harbored *lukSF-PV*, *lpl10*, *ssl1*, *sea*, *sek*, *seh*, *sec4*, *ear*, and *sel2*, some of the genes more frequently present in USA400 CA-MRSA strains (1, 40). Unlike the WSLH-6 isolate, MNDH-77 also harbored two of the *egc* genes (*seg* and *sei*) besides *eta*, *etb*, and *tst*.

DISCUSSION

This study presents the comparative analysis of genotypes and virulence profiles of 293 *S. aureus* isolates representing three major phenotypic groups of *S. aureus*—nasal carriage MSSA, clinical MSSA, and clinical CA-MRSA strains—collected over a 19-year period. Specifically, we determined the breadth of genotypic diversity for each group and assessed whether or not the virulence profile of CA-MRSA strains was significantly different from that of the MSSA groups. Genotyping using PFGE, MLST, and *spa* typing showed that the clinical and nasal carriage MSSA strains were highly diverse compared to the CA-MRSA group, which was found to be highly clonal. All CA-MRSA isolates belonged to the USA400 lineage only. This result was not entirely surprising as USA400 clones have primarily been reported from the midwestern United States during the collection period of 1990 to 1999 and continued to be reported from the midwestern United States and Alaska (8, 17, 40, 50, 52; S. K. Shukla et al., unpublished data). Unfortunately, we did not have USA300 strains in the study sample due to the time period (1990 to 1999) in which the gCA-MRSA isolates were collected. It should be noted that USA300, which was first identified in 2000, has now become the predominant CA-MRSA strain in most geographic areas in the United States, including the Midwest. Equally important was that we did not identify any clinical MSSA strains belonging to the USA300 clone in our collection.

Our study demonstrated that the virulence profile of the CA-MRSA USA400 strains was significantly different from

TABLE 5. Summary of the ranges of virulence gene positivity for each gene and percentages and numbers of gene positivity in each of the three groups along with the *P* values within each cohort

Gene	% of positivity range	*P* value[c]	% of positivity (no. of positives); *P* value		
			Nasal carriage[b]	Clinical MSSA[a]	CA-MRSA
Enterotoxin genes					
sea	54–95	—	58 (66); <0.0001	54 (56); <0.0001	95 (73)
seb	3–30	—	26 (29); <0.0001	30 (31); <0.0001	3 (2)
sec	25–95	—	27 (30); <0.0001	25 (26); <0.0001	95 (73)
sec4	25–97	—	29 (33); <0.0001	25 (26); <0.0001	97 (75)
sed	0–13	—	8 (9); 0.0116	13 (13); 0.0007	0 (0)
see	0–3	—	0 (0); 1.000	3 (3); 0.2615	0 (0)
seg	0–90	—	90 (102); <0.0001	89 (92); <0.0001	0 (0)
seg2	27–96	—	27 (31); <0.0001	31 (32); <0.0001	96 (74)
seh	15–100	—	15 (17); <0.0001	17 (17); <0.0001	100 (77)
sei	1–91	—	89 (101); <0.0001	91 (94); <0.0001	1 (1)
sej	1–9	—	9 (10); 0.0300	9 (9); 0.0452	1 (1)
sek	8–96	—	12 (14); <0.0001	8 (8); <0.0001	96 (74)
sel	9–96	—	9 (10); <0.0001	9 (9); <0.0001	96 (74)
sel2	25–97	0.0294	40 (45); <0.0001	25 (26); <0.0001	97 (75)
sem	0–47	—	42 (47); <0.0001	44 (45); <0.0001	0 (0)
sen	0–81	—	75 (85); <0.0001	81 (83); <0.0001	0 (0)
seo	0–82	—	76 (86); <0.0001	82 (84); <0.0001	0 (0)
Exotoxin genes					
ssl1	86–100	—	91 (103); 0.0061	86 (89); 0.0003	100 (77)
tst	0–78	0.0021	78 (88); <0.0001	58 (60); <0.0001	0 (0)
eta	0–56	—	54 (61); <0.0001	56 (58); <0.0001	0 (0)
etb	0–22	—	22 (25); <0.0001	18 (19); <0.0001	0 (0)
Leukocidin genes					
lukSF-PV	0–100	—	0 (0); <0.0001	3 (3); <0.0001	100 (77)
lukD	44–100	0.0010	66 (75); <0.0001	44 (45); <0.0001	100 (77)
lukE	62–100	0.0279	77 (87); <0.0001	62 (64); <0.0001	100 (77)
Hemolysin genes		—			
hla	100	—	100 (113); 1.0	100 (103); 1.0	100 (77)
hlb	91–100	0.0279	98 (111); 0.5154	91 (94); 0.0109	100 (77)
hld	100	—	100 (113); 1.0	100 (103); 1.0	100 (77)
hlgB	100	—	100 (113); 1.0	100 (103); 1.0	100 (77)
Surface protein genes					
clfA	70–99	—	76 (86); <0.0001	70 (72); <0.0001	99 (76)
clfB	97–100	—	97 (110); 0.2731	99 (102); 1.0	100 (77)
cna	95–100	0.0234	100 (113); 1.0	95 (98); 0.0721	100 (77)
fnbA	92–100	—	92 (104); 0.0116	95 (98); 0.0721	100 (77)
fnbB	0–24	—	24 (27); <0.0001	14 (14); 0.0003	0 (0)
icaA	99–100	—	100 (113); 0.4053	100 (103); 0.4278	99 (76)
lpl10	55–100	0.0171	55 (62); <0.0001	71 (73); <0.0001	100 (77)
sdrC	17–61	0.0008	82 (93); <0.0001	61 (63); <0.0001	17 (13)
sdrD	85–100	—	88 (100); 0.0010	85 (88); 0.0002	100 (77)
sdrE	90–100	—	96 (109); 0.1481	90 (93); 0.0054	100 (77)
Other genes		—			
bsa	100	—	100 (113); 1.0	100 (103); 1.0	100 (77)
ear	18–97	—	18 (20); <0.0001	21 (22); <0.0001	97 (75)

[a] *P* values were determined using CA-MRSA (reference) versus clinical MSSA.
[b] *P* values were determined using CA-MRSA (reference) versus nasal carriage isolates.
[c] *P* values were determined using clinical MSSA (reference) versus nasal carriage isolates. —, data not shown.

those of the nasal carriage and clinical MSSA isolates based on the presence of a number of pyrogenic superantigens and surface proteins in addition to the putative toxin genes identified in MW2. Fourteen of the 40 genes were significantly associated with the CA-MRSA group (Table 5). These genes included multiple enterotoxin genes, leukocidin genes, adhesion genes *sdrC* and *clfA*, and a few putative virulence genes. Specifically, all genes except the enterotoxin gene *see*; hemolysin genes *hla*, *hld*, and *hlgB*; and surface protein genes *clfB*, *cna*, and *icaA* (Table 5) had a higher rate of positivity in CA-MRSA USA400 isolates than in either clinical or nasal carriage MSSA isolates. The presence of additional virulence genes, particularly those that seem to provide redundant functions, could help to explain why strains of CA-MRSA USA400 are found to be more virulent than *S. aureus* strains in general. While expression of virulence genes is likely to be regulated by several factors,

TABLE 6. Comparison of virulence profiles of nasal carriage MSSA and clinical MSSA USA400 pulsed-field pulsotypes with three representative strains of USA400 CA-MRSA[a]

Isolate group and yr	Sample	*seh*	*ssl1*	*lpl10*	*sdrC*	*sdrD*	*sdrE*	*ear*	*sec*	*sec4*	*sel*	*sel2*	*clfa*	*hla*	*bsa*	*lukSF-PV*	*lukD*	*lukE*	*sea*	*seg2*	*sek*	*hlb*	*hld*	*hlgB*	*fnbB*	*fnbA*	*clfB*	*icaA*	*cna*	*seb*	*sed*	*see*	*seg*	*sei*	*sej*	*sem*	*sen*	*seo*	*eta*	*etb*	*tst*
Nasal MSSA																																									
2003	UWL-57-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2004	UWL-83-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2003	UWL-33-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2005	UWL-92-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2001	MNDH-125-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2000	MNDH-97-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Clinical MSSA																																								
1998	MNDH-77-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1987	WSLH-6-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2004	MC-112-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2002	WSLH-32-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2003	WSLH-35-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2004	MC-127-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1997	MNDH-31-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2004	MC-118-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CA-MRSA																																								
1992	34-R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1993	88-R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1994	169-R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] −, PCR negative; +, PCR positive.

including host factors, one can speculate that additional genes within the same functional class (e.g., toxins, adhesion factors, etc.) could provide a synergistic and/or complementary effect.

Several studies have described the evolution and virulence factor genes of HA-MRSA strains (15, 18); however, little has been done to examine these genes in CA-MRSA strains. Of the toxin-related virulence factor genes that have been examined in CA-MRSA strains, much has been done to characterize *lukSF-PV*, encoding the PVL. *lukSF-PV* has been shown to be conserved among most CA-MRSA isolates tested, but these same genes have been shown to be present in a low number of other *S. aureus* isolates (13, 30, 31, 47, 50, 57). Since this gene was present in only 1 to 5% of *S. aureus* strains in general but almost always in CA-MRSA USA400 strains isolated in the 1990s from the United States, it had become a molecular marker for USA400 isolates isolated during that time period (40, 50, 57). However, some recent studies from the United States and other parts of the world showed that the presence of PVL may not be the most definitive marker for recent CA-MRSA strains, including USA400 (32, 48, 55, 61). The results from our study found that 100% of CA-MRSA USA400 isolates and less than 1% of MSSA isolates possessed *lukSF-PV*, supporting previous studies (50, 57). The PVL has been shown to cause severe tissue damage and has been implicated in lung damage associated with strains causing severe necrotizing pneumonia (11, 19, 28, 31, 60). However, both a sepsis and an abscess model of infection with isogenic strains demonstrated that PVL-negative strains were as virulent as the PVL-positive parental strains (58). Thus, the role of PVL as a virulence factor is still under investigation. Besides PVL, α-hemolysin, α-type phenol-soluble modulins (PSMs), and the arginine catabolic mobile element (ACME) have been studied for their roles in the pathogenesis of CA-MRSA (9, 11). Lethal pneumonia may be tied to levels of α-hemolysin expressed in USA300 and USA400 strains (5). The PSMs expressed by USA300 and USA400 strains are thought to attack human neutrophils (59). Montgomery et al. (37), using pneumonia and the skin infection model in rabbits, showed that ACME is not associated with enhanced virulence. In contrast, Diep et al. (11) showed that deletion of ACME affects the pathogenicity and fitness of USA300. ACME, which was identified in the USA300 lineage, was not detected in 10 random samples of CA-MRSA strains from this study (data not shown).

Other toxins produced by *S. aureus* can also cause damage to host tissues. One class includes the staphylococcal pyrogenic exotoxins that can cause direct as well as indirect damage through overstimulation of the immune system (49). These exotoxins function as superantigens that keep the immune system from recognizing and attacking the invading *S. aureus*. A wide variety of toxins produced by *S. aureus* are considered to be pyrogenic exotoxins, including staphylococcal enterotoxins and toxic shock syndrome toxin (4).

Fourteen classical enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, and *seo*) have been previously identified in *S. aureus* and were screened for in this study (12, 23, 42, 43, 46, 62). The *sea*, *seb*, *sec*, *seh*, and *sek* genes have been reported to be found in the majority of CA-MRSA USA400 isolates from the United States collected prior to 2003 (17, 40), in contrast to USA400 isolates recovered during 2005-2006 (32). However, our results demonstrated that the CA-

MRSA isolates harbored a significantly higher percentage not only of the *sea*, *sec*, *seh*, and *sek* genes as expected but also of the *sec4*, *seg2*, *sel*, *sel2*, *lukSF-PV*, *lukD*, *lukE*, *clfA*, and *sdrD* genes than did either the nasal carriage or clinical MSSA groups. Interestingly, the clinical and nasal carriage MSSA groups possessed a significantly greater frequency of the *seb*, *sed*, *seg*, *sei*, *sej*, *sem*, *sen*, *seo*, *tst*, *eta*, *etb*, *fnbB*, and *sdrC* genes than did the CA-MRSA group. The higher frequency of *egc* in the MSSA cohorts and not in CA-MRSA strains suggests that they are acquired as part of *egc* as shown previously (24). A study by van Belkum et al. (56) determined that the *egc* genes were present in 63.7% of carriage isolates and 52.9% of invasive isolates. It is generally believed that the presence of this gene cluster is not associated with severe infections but probably helps in the carriage potential of an *S. aureus* strain (16, 22, 56). However, a case report of a severe case of necrotizing fasciitis in a leg of a diabetic patient that resulted in the amputation of the leg was caused by an MSSA strain that lacked most of the common virulence genes except the *egc* genes, suggesting that the products of *egc* may play a role, in some cases, in severe infections, especially in compromised patients (38). However, the proteins encoded by the *egc* genes seem to be produced in smaller amounts than are the other well-studied enterotoxins, inducing a lower immunologic response among human hosts (22) that may allow strains possessing the *egc* element to live a commensal lifestyle in healthy humans. In our study, we did not see any significant difference in the prevalence of *egc* between the carriage and clinical MSSA groups. The three other enterotoxin genes (*seb*, *sed*, and *sej*) were found in a much smaller percentage of the MSSA isolates but were almost always absent in CA-MRSA isolates. The *seb* gene encodes an enterotoxin that is known to be a potent superantigen (4, 49) that can overstimulate the host's immune system and be detrimental to a commensal lifestyle for the MSSA isolates. The function of *sed* and *sej* is not well established.

The success of the USA400 lineage of CA-MRSA in the midwestern United States and its enhanced virulence could be due to a number of reasons. First of all, this group of isolates harbors more enterotoxin and surface protein genes than do the clinical MSSA strains or the nasal carriage strains in the study. It has been shown that *sea* and *sec* are more frequently present in CA-MRSA than in HA-MRSA strains (17, 40), and septic shock appears to be associated with the presence of *sea* (16). Enterotoxins A and J along with virulence genes *fnbA*, *can*, *sdrE*, *sea*, *sej*, *eta*, *hlg*, and *ica* tend to be more common in invasive isolates (44). In a report by Kravitz et al., three of the five cases of purpura fulminans due to either MSSA or MRSA strains produced staphylococcal enterotoxin C (SEC) and PVL (27). SEA and SEC tend to produce higher immunological responses leading to host tissue damage (4, 49) than do other enterotoxins (16). In this context, the presence of the *sea* and *sec* genes along with other genes such *as seh*, *sek*, *sel*, *sel2*, *lukSF-PV*, *lukD*, *lukE*, *clfA*, and *sdrD* suggests that several virulence genes may be working in unison to facilitate or prolong an infection. It is also possible that the presence of multiple toxin and adhesion proteins could afford a redundancy of virulence arsenal to the pathogen to mount a more potent infection. Contradictory results of the virulence studies in animal models for PVL and ACME (10, 11, 19, 31, 37, 58) suggest that virulence in *S. aureus* is not likely to be associated with one toxin or surface protein but a cumulative or synergistic effect of several virulence factors to initiate, maintain, and prolong an infection. It is also very likely that the genomic background of a strain contributes to the overall virulence of an *S. aureus* strain (29). It was interesting that the nasal carriage and clinical MSSA isolates belonging to the USA400 genotype harbored one or more of the genes *egc*, *eta*, and *etb*, unlike the CA-MRSA isolates (Table 6). Only one of the USA400 MSSA strains harbored *fnbB*, typically found in USA300 strains. It is possible that a lack of these genes in the USA400 CA-MRSA strains, along with the gain of the SCC*mec* element, could have aided in the success of the CA-MRSA USA400 clone at the time in the midwestern United States. It is worth mentioning that now USA300 is the predominant CA-MRSA clone in most parts of the United States (54). Interestingly, introduction of USA300 in a correctional facility resulted in a clonal shift in the CA-MRSA clone, i.e., from USA400 to USA300 in that facility (51). This displacement of ST1:USA400 by the ST8:USA300 clone in different parts of the United States may be due to ACME in its genome, which allows enhanced survival on skin (9).

Although the roles that enterotoxins play in skin and soft tissue infections are not known, they have been shown to influence the development of secondary staphylococcal infections due to their proinflammatory effects that trigger increased inflammation (36). Although the other toxin genes have been described in *S. aureus*, their presence has not been tied to virulence. An exciting new finding that may offer some significant clues to the pathogenicity of the USA400 type of CA-MRSA was the presence of several new putative toxin genes (for example, *seg2*, *sel2*, *sec4*, *ear*, *lpl10*, and *ssl1*) in the CA-MRSA isolates that were present in lower percentages in both cohorts of MSSA strains. Each of the genes was found on pathogenicity islands on the genome of MW2 (1). Pathogenicity islands are distinct genetic elements that encode virulence factors found on bacterial genomes (21). The *seg2* gene was found on the ΦSa3 pathogenicity island, while the *lpl10* and *ssl1* genes were found on *v*Saα, and the *sel2*, *sec4*, and *ear* genes were found on a separate pathogenicity island labeled *v*Sa3. The functions of these genes have not yet been determined, and yet some sequence homology to the staphylococcal enterotoxins may help to elucidate the functions of the proteins encoded by the new genes (1). Furthermore, a proteomic study has shown antibodies to the Sel2 and Ear proteins in patients infected with CA-MRSA, suggesting a role for both proteins in CA-MRSA pathogenicity (6).

Acquisition of one or more of the new pathogenicity islands may play a role in CA-MRSA infections. Until now, there have been no studies that have looked at the distribution of these pathogenicity islands in CA-MRSA or any other *S. aureus* isolates. Our results show that the newly discovered toxin genes were found in most CA-MRSA isolates from the USA400 lineage. The MSSA cohorts displayed much smaller detection percentages for the *seg2*, *sel2*, *sec4*, and *ear* genes, while percentages for *ssl1* and *lpl10* were relatively higher in these groups but significantly less than those in the CA-MRSA cohort. These data suggest that the *v*Saα pathogenicity island (*ssl1* and *lpl10*) seems to be widely dispersed throughout *S. aureus* isolates; therefore, it is unlikely that the presence of this

island alone is responsible for the pathogenicity of CA-MRSA USA400 isolates. It seems more likely that the other islands are acting in unison to affect the pathogenicity of CA-MRSA isolates compared to the MSSA populations.

The presence of these genes among the CA-MRSA isolates and their absence among MSSA isolates suggest that the virulence of the CA-MRSA USA400 pulsed-field type may be highly dependent on this group of new putative toxin genes and that the virulence of this CA-MRSA type could be tied to the synergistic activities of the proteins encoded by the variety of these genes. Peacock et al. (44) postulated that the presence of certain virulence genes among *S. aureus* isolates is dependent upon the rates of gene acquisition and the cost to biological fitness of the organism itself.

In summary, our study showed that isolates belonging to the CA-MRSA USA400 lineage were genetically less diverse than the clinical and carriage MSSA isolates and harbored several additional virulence genes which may have helped them in efficient host acquisition and subsequent infection.

ACKNOWLEDGMENTS

We thank the Network on Antibiotic Resistance in *Staphylococcus aureus* and Jean Lee for supplying strains used as controls in this study. We also acknowledge Kathy Como-Sabetti, Jessica Nerby, and Dave Boxrud at the Minnesota Department of Health for their assistance with the MSSA isolates.

This work was supported by NIH grant AI061385 to S.K.S., the Marshfield Clinic Research Foundation, and a Graduate Student Research, Service, and Education Leadership Grant to M.E.K.

REFERENCES

1. **Baba, T., F. Takeuchi, M. Kuroda, H. Yuzawa, K. Aoki, A. Oguchi, Y. Nagai, N. Iwama, K. Asano, T. Naimi, H. Kuroda, L. Cui, K. Yamamoto, and K. Hiramatsu.** 2002. Genome and virulence determinants of high-virulence community-acquired MRSA. Lancet **359:**1819–1827.
2. **Bannerman, T. L., G. A. Hancock, F. C. Tenover, and J. M. Miller.** 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus* aureus. J. Clin. Microbiol. **33:**551–555.
3. **Becker, K., R. Roth, and J. Peters.** 1998. Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. J. Clin. Microbiol. **36:**2548–2553.
4. **Bohach, G. A., M. M. Dinges, D. T. Mitchell, D. H. Ohlendorf, and P. M. Schlievert.** 1997. Exotoxins, p. 83–111. *In* K. B. Crossley and G. L. Archer (ed.), The staphylococci in human disease. Churchill Livingstone, Inc., New York, NY.
5. **Bubeck Wardenburg, J., T. Bae, M. Otto, F. R. DeLeo, and O. Schneewind.** 2007. Poring over pores: alpha-hemolysin and Panton-Valentine leukocidin in *Staphylococcus aureus* pneumonia. Nat. Med. **13:**1405–1406.
6. **Burlak, C., C. H. Hammer, M. A. Robinson, A. R. Whitney, M. J. McGavin, B. N. Kreiswirth, and F. R. DeLeo.** 2007. Global analysis of community-associated methicillin-resistant *Staphylococcus* aureus exoproteins reveals molecules produced *in vitro* and during infection. Cell. Microbiol. **9:**1172–1190.
7. **Chambers, H. F.** 2001. The changing epidemiology of *Staphylococcus aureus?* Emerg. Infect. Dis. **7:**178–182.
8. **David, M. Z., K. M. Rudolph, T. W. Hennessy, S. Boyle-Vavra, and R. S. Daum.** 2008. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* rural southwestern Alaska. Emerg. Infect. Dis. **14:**1693–1699.
9. **Diep, B. A., S. R. Gill, R. F. Chang, T. H. Phan, J. H. Chen, M. G. Davidson, F. Lin, J. Lin, H. A. Carleton, E. F. Mongodin, G. F. Sensabaugh, and F. Perdreau-Remington.** 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. Lancet **367:**731–739.
10. **Diep, B. A., A. M. Palazzolo-Balance, P. Tattevin, L. Basuino, K. R. Braughton, A. R. Whitney, L. Chen, B. N. Kreiswirth, M. Otto, F. R. DeLeo, and H. F. Chambers.** 2008. Contribution of Panton-Valentine leukocidin in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. PLoS One **3:**e3198.
11. **Diep, B. A., G. G. Stone, L. Basuino, C. J. Graber, A. Miller, S. A. des Etages, A. Jones, A. M. Palazzolo-Balance, F. Perdreau-Remington, G. F. Sensabaugh, F. R. DeLeo, and H. F. Chambers.** 2008. The arginine catabolic mobile element and staphylococcal chromosomal cassette *mec* linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant *Staphylococcus aureus*. J. Infect. Dis. **197:**1523–1530.
12. **Dinges, M. M., P. M. Orwin, and P. M. Schlievert.** 2000. Exotoxins of *Staphylococcus aureus*. Clin. Microbiol. Rev. **13:**16–34.
13. **Dufour, P., Y. Gillet, M. Bres, G. Lina, F. Vandenesch, D. Floret, J. Etienne, and H. Richet.** 2002. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. Clin. Infect. Dis. **35:**819–824.
14. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
15. **Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt.** 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. U. S. A. **99:**7687–7692.
16. **Ferry, T., D. Thomas, A. L. Genestier, M. Bes, G. Lina, F. Vandenesch, and J. Etienne.** 2005. Comparative prevalence of superantigen genes in *Staphylococcus aureus* isolates causing sepsis with and without septic shock. Clin. Infect. Dis. **41:**771–777.
17. **Fey, P. D., B. Said-Salim, M. E. Rupp, S. H. Hinrichs, D. J. Boxrud, C. C. Davis, B. N. Kreiswirth, and P. M. Schlievert.** 2003. Comparative molecular analysis of community-or hospital-acquired methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **47:**196–203.
18. **Fitzgerald, J. R., D. E. Sturdevant, S. M. Mackie, S. R. Gill, and J. M. Musser.** 2001. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. Proc. Natl. Acad. Sci. U. S. A. **98:**8821–8826.
19. **Gillet, Y., B. Issartel, P. Vanhems, J. C. Fournet, G. Lina, F. Vandenesch, Y. Piedmont, N. Brousse, D. Floret, and J. Etienne.** 2002. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotizing pneumonia in young immunocompetent patients. Lancet **359:**753–758.
20. **Grundmann, H., S. Hori, M. C. Enright, C. Webster, A. Tami, E. J. Feil, and T. Pitt.** 2002. Determining the genetic structure of natural population of *Staphylococcus aureus*: a comparison of multilocus sequence typing with pulsed-field gel electrophoresis, randomly amplified polymorphic DNA analysis, and phage typing. J. Clin. Microbiol. **40:**4544–4546.
21. **Hacker, J., and J. B. Kaper.** 2000. Pathogenicity islands and the evolution of microbes. Annu. Rev. Microbiol. **54:**641–679.
22. **Holtfreter, S., K. Bauer, D. Thomas, C. Feig, V. Lorenz, K. Roschack, E. Friebe, K. Selleng, S. Lovenich, T. Greve, A. Greinacher, B. Panzig, S. Engelmann, G. Lina, and B. M. Broker.** 2004. *egc*-encoded superantigens from *Staphylococcus aureus* are neutralized by human sera much less efficiently than are classical staphylococcal enterotoxins or toxic shock syndrome toxin. Infect. Immun. **72:**4061–4071.
23. **Jarraud, S., C. Mougel, J. Thioulouse, G. Lina, H. Meugnier, F. Forey, X. Nesme, J. Etienne, and F. Vandenesch.** 2002. Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect. Immun. **70:**631–641.
24. **Jarraud, S., M. A. Peyrat, A. Lim, A. Tristan, M. Bes, C. Mougel, J. Etienne, F. Vandenesch, M. Bonneville, and G. Lina.** 2001. *egc*, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. J. Immunol. **166:**669–677.
25. **Klein, E., D. L. Smith, and R. Laxminarayan.** 2009. Community-associated methicillin-resistant *Staphylococcus aureus* in outpatients, United States, 1999–2006. Emerg. Infect. Dis. **15:**1925–1930.
26. **Koreen, L., S. V. Ramaswamy, E. A. Graviss, S. Naidich, J. M. Musser, and B. N. Kreiswirth.** 2004. *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J. Clin. Microbiol. **42:**792–799.
27. **Kravitz, G. R., D. J. Dries, M. L. Peterson, and P. M. Schlievert.** 2005. Purpura fulminans due to *Staphylococcus aureus*. Clin. Infect. Dis. **40:**941–947.
28. **Labandeira-Rey, M., F. Couzon, S. Boisset, E. L. Brown, M. Bes, Y. Benito, E. M. Barbu, V. Vazquez, M. Hook, J. Etienne, F. Vandenesch, and M. G. Bowden.** 2007. *Staphylococcus aureus* Panton-Valentine leukocidin causes necrotizing pneumonia. Science **315:**1130–1133.
29. **Li, M., B. A. Diep, A. E. Villaruz, K. R. Braughton, X. Jiang, F. R. DeLeo, H. F. Chambers, Y. Lu, and M. Otto.** 2009. Evolution of virulence in epidemic community-associated methicillin-resistant *Staphylococcus aureus*. Proc. Natl. Acad. Sci. U. S. A. **106:**5883–5888.
30. **Liassine, N., R. Auckenthaler, M. C. Descombes, M. Bes, F. Vandenesch, and J. Etienne.** 2004. Community-acquired methicillin-resistant *Staphylococcus aureus* isolated in Switzerland contains the Panton-Valentine leukocidin or exfoliative toxin genes. J. Clin. Microbiol. **42:**825–828.
31. **Lina, G., Y. Piedmont, F. Godail-Gamot, M. Bes, M. O'Peter, V. Gauduchon, F. Vandenesch, and J. Etienne.** 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. **29:**1128–1132.
32. **Limbago, B., G. E. Fosheim, V. Schoonover, C. E. Crane, J. Nadle, S. Petis,**

D. Heltzel, S. M. Ray, L. H. Harrison, R. Lynfield, G. Dumyati, J. M. Townes, W. Schaffner, Y. Mu, and S. K. Fridkin for the Active Bacterial Core Surveillance (ABCs) MRSA Investigators. 2009. Characterization of methicillin-resistant *Staphylococcus aureus* isolates collected in 2005-2006 from patients with invasive disease: a population-based analysis. J. Clin. Microbiol. **47:** 1344–1351.

33. **Lowy, F. D.** 1998. *Staphylococcus aureus* infections. N. Engl. J. Med. **339:** 520–532.
34. **McDougal, L. K., C. D. Steward, G. E. Killgore, J. M. Chaitram, S. K. McAllister, and F. C. Tenover.** 2003. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J. Clin. Microbiol. **41:**5113–5120.
35. **McDougal, L. K., Z. Wenming, J. B. Patel, and F. C. Tenover.** 2004. Characterization of two new community-associated oxacillin resistant *Staphylococcus aureus* pulsed-field types consisting of US isolates that carry SCC*mec* IV and Panton-Valentine leukocidin genes, abstr. C-220, p. 163. Abstr. 104th Gen. Meet. Am. Soc. Microbiol.
36. **Mempel, M., G. Lina, M. Hojka, C. Schnopp, H. P. Seidle, T. Schafer, J. Ring, F. Vandenesch, and D. Abeck.** 2003. High prevalence of superantigen associated with the *egc* locus in *Staphylococcus aureus* isolated from patients with atopic eczema. Eur. J. Clin. Microbiol. Infect. Dis. **22:**306–309.
37. **Montgomery, C. P., S. Boyle-Vavra, and R. S. Daum.** 2009. The arginine catabolic mobile element is not associated with enhanced virulence in experimental invasive disease caused by the community-associated methicillin-resistant *Staphylococcus* aureus USA300 genetic background. Infect. Immun. **77:**2650–2656.
38. **Morgan, W. R., M. D. Caldwell, J. M. Brady, M. E. Stemper, K. D. Reed, and S. K. Shukla.** 2007. Necrotizing fasciitis due to a methicillin-sensitive *Staphylococcus aureus* harboring an enterotoxin gene cluster. J. Clin. Microbiol. **45:**668–671.
39. **Munson, S. H., M. T. Tremaine, M. J. Betley, and R. A. Welch.** 1998. Identification and characterization of staphylococcal enterotoxin types G and I from *Staphylococcus aureus*. Infect. Immun. **66:**3337–3348.
40. **Naimi, T. S., K. H. LeDell, K. Como-Sabetti, S. M. Borchardt, D. J. Boxrud, J. Etienne, S. K. Johnson, F. Vandenesch, S. Fridkin, C. O'Boyle, R. N. Danila, and R. Lynfield.** 2003. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA **290:** 2976–2984.
41. **Oliveira, D. C., and H. de Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **46:** 2155–2161.
42. **Orwin, P. M., D. Y. Leung, H. L. Donahue, R. P. Novick, and P. M. Schlievert.** 2001. Biochemical and biological properties of staphylococcal enterotoxin K. Infect. Immun. **69:**360–366.
43. **Orwin, P. M., J. R. Fitzgerald, D. Y. Leung, J. A. Gutierrez, G. A. Bohach, and P. M. Schlievert.** 2003. Characterization of *Staphylococcus aureus* enterotoxin L. Infect. Immun. **71:**2916–2919.
44. **Peacock, S. J., C. E. Moore, A. Justice, M. Kantzanou, L. Story, K. Mackie, G. O'Neill, and N. P. J. Day.** 2002. Virulent combinations of adhesion and toxin genes in natural populations of *Staphylococcus aureus*. Infect. Immun. **70:**4987–4996.
45. **Popovich, K. J., R. A. Weinstein, and B. Hota.** 2008. Are community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains? Clin. Infect. Dis. **46:**787–794.
46. **Ren, K., J. D. Bannan, V. Pancholi, A. L. Cheung, J. C. Robbins, V. A. Fischetti, and J. B. Zabriske.** 1994. Characterization and biological properties of a new staphylococcal exotoxin. J. Exp. Med. **180:**1675–1683.
47. **Robinson, D. A., and M. C. Enright.** 2003. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **47:**3926–3934.
48. **Rossney, A. S., A. C. Shore, P. M. Morgan, M. M. Fitzgibbon, B. O'Connell, and D. C. Coleman.** 2007. The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Panton-Valentine leukocidin gene (*pvl*) reveal that *pvl* is a poor marker for community-acquired MRSA strains in Ireland. J. Clin. Microbiol. **45:**2554–2563.
49. **Schlievert, P. M.** 1995. Role of superantigens in human diseases. Curr. Opin. Infect. Dis. **8:**170–174.
50. **Shukla, S. K., M. E. Stemper, S. V. Ramaswamy, J. M. Conradt, R. Reich, E. A. Graviss, and K. D. Reed.** 2004. Molecular characteristics of nosocomial and native American community-associated methicillin-resistant *Staphylococcus aureus* clones from rural Wisconsin. J. Clin. Microbiol. **8:**3752–3757.
51. **Stemper, M. E., J. M. Brady, S. Qutaishat, G. Borlaug, J. Reed, K. D. Reed, and S. K. Shukla.** 2006. Shift in *Staphylococcus aureus* clone linked to an infected tattoo. Emerg. Infect. Dis. **12:**1444–1446.
52. **Stemper, M. E., S. K. Shukla, and K. D. Reed.** 2004. Emergence and spread of community-associated methicillin-resistant *Staphylococcus aureus* in rural Wisconsin, 1989–1999. J. Clin. Microbiol. **42:**5673–5680.
53. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
54. **Tenover, F. C., and R. V. Goering.** 2009. Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology. J. Antimicrob. Chemother. **64:**441–459.
55. **Tsigrelis, C., M. D. Armstrong, N. E. Vlahakis, J. A. Batsis, and L. M. Baddour.** 2007. Infective endocarditis due to community-associated methicillin-resistant *Staphylococcus aureus* in injection drug users may be associated with Panton-Valentine leukocidin-negative strains. Scand. J. Infect. Dis. **39:**299–302.
56. **van Belkum, A., D. C. Melles, S. V. Snijders, W. B. van Leeuwen, H. F. L. Wertheim, J. L. Nouwen, H. A. Verbrugh, and J. Etienne.** 2006. Clonal distribution and differential occurrence of the enterotoxin gene cluster, *egc*, in carriage- versus bacteremia-associated isolates of *Staphylococcus aureus*. J. Clin. Microbiol. **44:**1555–1557.
57. **Vandenesch, F., T. Naimi, M. C. Enright, G. Lina, G. R. Nimmo, H. Heffernan, N. Liassine, M. Bes, T. Greenland, M. Reverdy, and J. Etienne.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis. **9:**978–984.
58. **Voyich, J. M., M. Otto, B. Mathema, K. R. Braughton, A. R. Whitney, D. Welty, R. D. Long, D. W. Dorward, D. J. Gardner, G. Lina, B. N. Kreiswirth, and F. R. DeLeo.** 2006. Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? J. Infect. Dis. **194:**1761–1770.
59. **Wang, R., K. R. Braughton, D. Kretschmer, T. H. Bach, S. Y. Queck, M. Li, A. D. Kennedy, D. W. Dorward, S. J. Klebanoff, A. Peschel, F. R. DeLeo, and M. Otto.** 2007. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat. Med. **13:**1510–1514.
60. **Yamasaki, O., J. Kaneko, S. Morizane, H. Akiyama, J. Arata, S. Narita, J. Chiba, Y. Kamio, and K. Iwatsuki.** 2005. The association between *Staphylococcus aureus* strains carrying Panton-Valentine leukocidin genes and the development of deep-seated follicular infection. Clin. Infect. Dis. **40:**381–385.
61. **Zhang, K., J. A. McClure, S. Elsayed, J. Tan, and J. M. Conly.** 2008. Coexistence of Panton-Valentine leukocidin-positive and -negative community-associated methicillin-resistant *Staphylococcus aureus* USA400 sibling strains in a large Canadian health-care region. J. Infect. Dis. **197:**195–204.
62. **Zhang, S., J. J. Iandolo, and G. C. Stewart.** 1998. The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (*sej*). FEMS Microbiol. Lett. **168:**227–233.